DIFFERENTIATION BY SEGREGATION AND ENVIRONMENT IN THE DEVELOPING ORGANISM[1]

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BIOLOGICAL investigations in the twentieth century have markedly strengthened the belief in the specificity of different kinds of living matter. Paleontology has shown the existence of organisms which have retained their specificity during millions of years: specific germplasma has carried through ages specific characters. On the other hand discoveries in the world of microorganisms have shown, that even their simplest forms are characterized if not always by specific organization, at least by definite metabolism and other biological qualities which imply a specificity of their constitution.

I shall not discuss the problem of genus specificity. My subject is limited to the specificity of certain tissues and cells found in the organism, the final development of which results in the symbiosis of differently organized tissues. The problem whether the relations of these different tissues is definitely determined by their specificity, or whether there exist in the organism plastic factors which from a homogeneous cell material may mould differently organized products is still unsettled. The solution of this problem would be greatly advanced, if the results of experimental and descriptive histogenesis received due consideration. Though the microscope can indeed not distinguish between various colloidal solutions, it might and does give data of definite biological significance.

Different genera and species show under the microscope a different structure of their building stones—the

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cells, and most conspicuously in their chromosome-complexes. The specificity manifested by genus and species, whether it is centered in specific proteins of the cytoplasm or in specific molecules of the chromosomes, forms one great chapter of the specificity problem, while the specificity of different tissues and cells is another.

It is often stated that the different tissues and cells of an individual of a given species, all have identical chromosome-complexes. If the chromosomes are considered identical in all the cells of an individual, they can not be regarded as responsible for the specificity of his tissues. They can place no restriction upon a wide range of permutability between the various cells in the organism, can put no restraint upon unlimited regeneration or impede the perpetual proliferation of any type of cells. The assumption of equality of chromosome-complexes in different tissues and of their invariability excludes them from the range of possible carriers of the specificity of tissues and is usually associated with the belief that the specificity of tissues is brought about by segregation of cytoplasmic materials during development. The possibility is also considered, that environment may act as differential factor.

Of these two latter factors the segregation of cytoplasmic materials in the early stage of development leads to the formation of large cell groups (germ-layers, anlages of organs), the differential characters of which are believed to be determined by the presence of definite cytoplasmic materials, transferred to them from the cytoplasm of the ovum. The differentiation brought about by segregation is regarded as irreversible and though the cells of the germ-layers show a great plasticity in their response to different factors, there is a well-marked limitation of their potencies, if compared with the first blastomeres. It is believed, however, that the segregation does not affect the chromosomes and produces merely a differential distribution of the cytoplasmic constituents of the ovum among the resulting cell groups. It has been

recently shown that at least the embryonic mesenchymal cells have an unlimited power of regeneration and therefore can be considered potentially immortal.

In passing I should like to point out that the uninterrupted synthesis of the chromatin during cell proliferation may be secondarily influenced by the differences in the cytoplasm thus acquired. The assumption of invariability on the part of the chromosome-complexes would imply a further assumption of persistence in the cytoplasm at least of some unchanged metabolic processes identical for all cells, to which the synthesis of identical chromatin could be referred.

Differentiation by segregation is a fact proved experimentally and many striking examples of segregation of various cytoplasmic materials during cleavage are found in Wilson's and Conklin's work. As result of segregation a number of cell groups appear. The groups are different, but the cells of each of them are similar. The various cytoplasmic substances distributed to the cell groups are specific, can not be built up by cells, which do not contain them and influence the further development of the cells in a definite manner.

These groups of cells proliferate and differentiate, giving rise to a number of specific organs, tissues and cells. Does a further segregation of definite substances continue at the time of the final specialization of tissues, are the cell potentialities gradually narrowed by further differential distribution of cytoplasmic constituents, and finally rendered univalent and irreversible? Leaving aside the question as to how specific tissues arise from specific anlages, Loeb in his last book, an important and stimulating publication, adopts the view shared also by Stockard, of the specificity of anlages in the organism. *The anlages are,* in Loeb's conception, *"destined to give rise to definite organs."* He considers "the formation of the various organs of the body, as being due to the development of *specific cells* in definite locations in the organism, *which will grow out into definite organs, no*

matter into which part of the organism they are transplanted." This assumption may apply to a number of developmental processes in the organism, but the statement is only part of the truth. Years of study of the loose mesenchyme and of the differential processes, observed in this tissue, have yielded a few results, which most decidedly do not harmonize with the generalization above quoted.

The loose mesenchyme, which appears in the early stages, is characterized by its ubiquity and by lack of obvious special function, if its mere presence between other organs has not to be considered as function. The mesenchyme is a syncytium of similar cells, the structure and most probably the metabolism of which is little if at all changed, while the cells remain as constituent parts of the syncytium. Influences of local origin which might change the metabolism of some of the cells are inhibited by continuous unimpeded flow and intermixture of substances in the undivided bodies of the cells.

Cells of the loose mesenchyme become isolated from the syncytium in many parts of the organism. This process of isolation is diffuse, in some parts of the organism it affects merely a small number of cells, in others it is displayed with great intensity. Scattered free cells or large groups of them are formed. The cause of such *isolation?* If it is not possible to formulate it in positive terms, at least it can be stated, that *it does not depend upon predestination,* centered in the syncytium itself, since isolation of cells from a mesenchymal syncytium can be greatly intensified experimentally. Large groups of free cells develop in the embryo after certain grafts on its allantois in regions in which normally the cells would retain their syncytial connections.

The free ameboid cells isolated from the mesenchymal syncytium differ from the cells of their maternal basis in many respects. Their metabolism is no longer controlled and regulated by the metabolism of the whole colony of mesenchymal tissue. Isolated they are very active, grow

intensely, frequently divide and their structure undergoes rapidly a series of changes which are not always identical and which transform them into various blood cells. Do these various changes exclusively depend upon the physicochemical constitution of the cells, in other words are they predestined, will each of these cells grow into a definite unit, no matter to what condition it is subjected? Is the group of ameboid, morphologically similar cells freed from the mesenchymal syncytium still formed by a number of species cells, the characteristics of which consist if not in a discernible structure, yet in an inherent necessity to develop along definite lines?

A series of investigations, some of them my own, have pointed to the group of the free ameboid cells as the mother cells of various blood elements. In regions where the isolation is merely occasional, scattered wandering cells arise. In regions where the isolation of free cells is intense, so-called anlages of hematopoietic organs develop. The first stages of development of various hematopoietic organs were found to be much alike and the continuous differentiation of the various blood cells throughout life was shown to have for its starting point a cell, the structure of which is similar to that of the ameboid cell, which arise from the mesenchyme.

Moreover, it was observed that there existed an invariable *association between the development of the mother cell into a definite blood cell and definite environmental conditions,* viz., if left in the spaces amongst mesenchymal cells the free ameboid cell develops into a granuloblast, especially in the vicinity of thin walled vessels; if surrounded by endothelial walls and subjected to intravascular conditions it develops into an erythroblast. This association has been established in the hematopoiesis of birds, reptiles, amphibia and certain fishes. The association between differentiation of the stem cell and environment on account of the regularity with which it was observed suggested to me the idea, that it was more than mere coincidence, and that possibly environment contained the differential fac-

tors, which from a homogeneous cell material moulded different products.

On the basis of descriptive histogenetic studies it seemed plausible to admit that environment can modify isolated cells; that the metabolic processes of the cells are the resultant of their physico-chemical constitution plus physico-chemical conditions of the environment (of course hormons, enzymes and so forth are included in the environment) and do not depend exclusively upon their physico-chemical constitution; that different substances arise in the cell-body (hemoglobin, various specific granules) in polyvalent cells as result of changes, determined by differences in the environment. The existence of cells endowed with various potencies has in consequence been largely admitted. The specificity of the various mature blood cells would thus be brought about by factors extrinsic to the stem cells.

These conclusions are based on facts established by descriptive histogenetic studies. Experimental proofs are beginning to accumulate which soon will leave no doubt of the validity of these conclusions. The existence of polyvalent cells would be proved, if, for example, hemoblasts subjected to various conditions would undergo various differentiation. If stem cells from within the vessels were transferred into the spaces between the mesenchymal cells and here instead of developing into erythroblasts, differentiated into granuloblasts (these experiments are under way) the stem cells within the vessels would be proved to be polyvalent. The same applies to other blood cells. As recently shown, spleenic follicles, the cells of which normally differentiate into small lymphocytes, if grafted on the chick allantois resolve themselves into numerous hemoblasts, which finally undergo a granuloblastic differentiation and give typical granular leucocytes. Thus the results of the histogenetic studies by experimental method entail the recognition in the embryo and in the adult organism of tissues and cells, which have not been fully differentiated and remain polyvalent.

It is the polyvalent cells which are the source of the wide range of regeneration encountered, particularly in the lower animals. It is astonishing to see how readily students of differentiation and specificity reconcile the extensive regeneration observed in many organisms with the belief in the specificity of the anlages of organs. Driesch has shown that gills excised from an Ascidian can regenerate a whole animal with heart, intestine and stolon. If in the particular case the anlages of the gills and the gills themselves were built of specific cells, the results of the experiment would be inconsistent. How could heart, intestine and stolon regenerate from the gills if the cells of the gills were not endowed with various potencies; if specific, they would grow only into the same tissue under all conditions. On what other basis could the experiments of Child's be explained, in which cells of a definite segment in the *Planaria* will regenerate a head or a tail, according to whether it formed the anterior or the posterior part of the piece cut out from the worm? The very fact that different specific structures may be regenerated at the expense of one common source, as, for example, heart and intestine from a gill or erythrocytes, granulocytes and small lymphocytes from hemoblasts, implies the polyvalency of their common source.

It is known, indeed, that environment can educe new qualities in the organism, but they usually subsist only while the specific conditions are present, and are lost if the organism is transferred to another environment. Such changes are not specific. The changes revealed by the freed mesenchymal cells, which result in the formation of mature blood cells, would only then be called specific, if they were retained by generations of their descendants under different conditions. An indifferent hemoblast within the vessels is soon transformed into an erythroblast, which shows in its cytoplasm the first traces of hemoglobin. Is the erythroblast a definitively specific cell, univalent and no longer capable of heteroplastic differentiation in new environment? New environmental condi-

tions for an erythroblast can be found in the organism outside the vessels, where hemoblasts develop into granuloblasts or small lymphocytes. If transplanted outside the vessels, the erythroblasts still developed further into analogous cells, this would mean that the changes which inside the vessels have transformed a polyvalent hemoblast into an erythroblast are irreversible (at least in the organism), that they have narrowed the potencies of the erythroblast in comparison with its mother cell and have rendered it specific, *i. e.* univalent and irreversible in its metabolism. Positive results from such experiment, could they be attained, would be of great value; they would prove that definite factors encountered in the normal organism outside of a cell call forth such changes as would be transmitted by the cell to its daughter cells even if the differential factors had no longer direct influence upon them.

The arrangement of such experiments offers however insuperable difficulties. Hemoblasts or mesenchymal cells can be transplanted, for there are stages in the hematopoiesis of the yolk-sac, in which capillaries are distended exclusively by hemoblasts and at this time they can be transferred into the spaces between the mesenchymal cells. It would be hardly possible to pick out from within the vessels erythroblasts, in which hemoglobin had already begun to develop, but which still were capable of proliferation. Most fortunately the required experiment has been carried out in a series of allantois by nature herself.

The grafting on the allantois which I used in my recent work is often accompanied by an extensive edema in the mesenchyme, which also affected the endothelium of the vessels. At the time of grafting (seventh to eighth day of incubation) the vessels contain numerous young erythroblasts, which after grafting become particularly numerous. The loosening of the vascular wall made it possible for a number of erythroblasts to escape from within the vessels. As a result of these conditions large

groups of cells appeared in the spaces between the mesenchymal cells, which already had begun their erythroblastic differentiation, while within the vessels. These cells, now outside the vessels, proliferate and continue their differentiation into erythroblasts, and their cytoplasm is gradually transformed into or substituted by homogeneous hemoglobinic substance.

The changes undergone by a polyvalent hemoblast within the vessels are thus no longer reversible outside of them. The differentiation determined by environmental conditions has been rendered specific, *i. e.*, univalent and irreversible. The specificity of tissue and cells can not therefore be the result alone of segregation of different cytoplasmic materials during cleavage. The process of segregation, of course, transfers different materials to different cell groups, the presence of which impedes their permutability, but these cell groups are still polyvalent and may, under various conditions, undergo various development.

The relations between these cell groups, the structures, effected by them, the different products of their metabolism, form the external factors of the environment which gradually render the cells of a polyvalent group specific, univalent and irreversible in their potencies. This specificity is transmitted by mother cells to their daughter cells irrespectively of the environmental conditions, to which they are subjected.

A few words concerning the structural changes of the cells during their definitive specialization. Differentiation during cleavage is effected by transmission of different cytoplasmic materials to different cell groups. What kind of changes in the cell structure are induced by the external factors of the environment? Compare the structure of the mature univalent blood cells with that of their mother cells in the stage of a hemoblast. Cytoplasm, structure of the resting nucleus, chromosome-complexes during mitosis, as demonstrated by our microscopical preparation, have undergone such fundamental changes,

as to have required thorough and detailed investigations in order to establish their reciprocal relationship. The size of the cells makes difficult a detailed study of the changes in the chromosomes, and they require further investigation, nevertheless the possibility of distinguishing different types of chromosome-complexes in different cells is not to be overlooked; it is easy to identify, for example, in the thymus entodermal cells, hemoblasts and small lymphocytes, during mitosis by their chromosome-complexes. The assumption of invariability on the part of the chromosome-complexes in the somatic cells requires some qualification. The chromosomes of a cell and the cytoplasm together embody specificity. Changes in both may transform the cell so completely as to deprive it of its faculty of proliferation. Erythrocytes and leucocytes in the blood cell series afford examples of such final modifications which have been gradually determined at least in part by the external factors of the environment.